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MOVEMENT OF MERCURY IN RAT SUBMAXILLARY SLICES*,**

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SUMMARY

The movement of $^{203}Hg^{2+}$ (as $HgCl_2$) was studied in slices of rat submaxillary gland. A steady-state distribution was reached within 60 min. N_2 or metabolic inhibitors (2,4-DNP, NaCN, NaF or ouabain) failed to decrease tissue uptake. Increasing the concentration of Hg^{2+} produced a corresponding increase in tissue metal. Decreasing the incubation temperature decreased uptake. Cd^{2+} and Fe^{2+} decreased uptake but Zn^{2+}, Cu^{2+}, and Fe^{3+} were without effect. Efflux experiments indicated a firm binding of Hg^{2+}. The data suggest a passive uptake for Hg^{2+} with unsaturable, temperature-sensitive binding sites in the interior of the cell.

INTRODUCTION

Mercury has been identified in the blood, urine and saliva of occupationally-exposed humans [1,2]. Joselow et al. [3] further examined the relationships between blood, urinary and salivary mercury in humans and suggested the use of saliva as an indicator of trace metal absorption. In addition, mecury has been demonstrated in the salivary glands of mice which received single injections of $HgCl_2$ [4,5].

Mercury is present in rat salivary tissue and is concentrated in rat saliva relative to blood filtrate [6]. The experiments reported in this paper were designed to examine the mechanisms of mercury transport.

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16. ABSTRACT

The movement of $^{203}Hg^{2+}$ (as $HgCl_2$) was studied in slices of rat submaxillary gland. A steady-state distribution was reached within 60 min. N_2 or metabolic inhibitors (2,4-DNP, NaCN, NaF or ouabain) failed to decrease tissue uptake. Increasing the concentration of Hg^{2+} produced a corresponding increase in tissue metal. Decreasing the incubation temperature decreased uptake. Cd^{2+} and Fe^{2+} Decreased uptake but Zn^{2+}, Cu^{2+}, and Fe^{3+} were without effect. Efflux experiments indicated a firm binding of Hg^{2+}. The data suggest a passive uptake for Hg^{2+} with unsaturable, temperature-sensitive binding sites in the interior of the cell.

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METHODS

Experiments were performed according to Putney and Borzelleca [7]. Adult male Sprague—Dawley rats (250—350 g, Flow Labs) were anesthetized with urethane (1200 mg/kg, J.T. Baker Chemical Co.). The submaxillary glands were removed and placed in previously-oxygenated incubation medium (modifed Krebs-Ringer-Tris-phosphate buffer, pH 7.4). Thin slices (40—70 mg, 0.4—0.5 mm) were prepared with a Stadie-Riggs microtome. Each slice was placed in a polyethylene support (in 5 ml of medium, in a 20 ml glass vial) in a constant temperature water bath. The support, fashioned from a disposable syringe barrel, functioned as a holder for the tissue slice and facilitated transferral of the slice between vials.

$^{203}HgCl_2$ (New England Nuclear) was diluted with $HgCl_2$ (Mallinckrodt Chemical Works) to a final concentration of 1×10^{-6} M $^{203}Hg^{2+}$ (0.05 μCi/ml). Solutions were prepared in 0.9% NaCl made with distilled deionized water.

Uptake experiments consisted of 60-min equilibration in medium followed by 60-min incubation with radiolabeled metal under various conditions. Standard conditions were 10^{-6} M $^{203}Hg^{2+}$ (pH 7.4), 37°C and 100% O_2. After incubation, each slice was removed from the experimental medium, rinsed 4 times with fresh medium, blotted, weighed and digested overnight in NaOH. HCl was added and aliquots of the tissue digest and medium were spotted on planchets and assayed on a Nuclear Chicago low background planchet counter. Counts (cpm) were corrected for quenching by the tissue digest. Preliminary experiments were conducted to determine the appropriate multiplication factor (1.80 ± 0.19, $n = 18$). Uptake was determined by proportion; nmol tissue metal were calculated from aliquots of the tissue digest and incubation medium. This was corrected for counting efficiency of the radionuclide detector and for different specific activities in the experimental medium. Results are expressed as nmol Hg^{2+}/g wet weight tissue.

Tissue respiration was determined under similar conditions, with non-radioactive metal. Slices were equilibrated for 60 min and then incubated for an additional 60 min and 120 min with various concentrations of Hg^{2+}. The slices were temporarily removed from the supports at 30, 60, 120 and 180 min and placed into 5 ml of fresh medium in a 25-ml cylindrical glass vial. Oxygen consumption was measured over a 2-min period with a Clark polarographic electrode at 37°C as a decrease from 100% saturation of the medium. No significant washout (removal) of mercury was seen. The 30-min and 60-min equilibration readings (for each Hg^{2+} concentration tested) were averaged and taken as 100% O_2 consumption. Readings at 120 min and 180 min were then expressed as the % of first-hour respiration.

For efflux experiments, slices were transferred, after incubation, through a series of 25 vials (each with 5 ml of medium) for 2-min intervals. Each slice was digested as above and the radioactivity was determined in each washout vial and in the tissue digest. Data were computed as the % radioactivity remaining in the tissue with time and were plotted semilogarithmically to yield

desaturation curves [8]. Rate constants were calculated as the mean of the individual slopes for the first-order curves between adjacent experimental points.

Student's *t*-test for unpaired means was used for comparison of 2 groups [9]. Significance was judged at the 0.05 level. Rectilinear curves of best fit were determined by linear regression.

RESULTS

Inhibition of O_2 consumption (30—50%) was evident with 10^{-4} M Hg^{2+}; the stimulation at low concentrations (10^{-6} and 10^{-5} M) was not significant (Fig. 1).

A steady-state distribution was reached by 60 min with 10^{-6} M Hg^{2+}; similar behavior was suggested with 10^{-4} M (Fig. 2).

Proportional increases in uptake (r = 0.99) were observed with 10-fold increases in metal concentration (Table I). Incubation at 25°C depressed uptake by 3.9 nmol Hg^{2+}/g. Incubation at 25°C further depressed uptake by 5.5 nmol Hg^{2+}/g. This is approximately 3.0 nmol Hg^{2+}/g/10°C. Raising or lowering the pH produced insignificant changes in uptake. While the increase in uptake at pH 6.8 was marked, variability of the response excluded statistical significance. Since Hg^{2+} was used as the chloride salt, the metal should be ionized in the incubation medium. Hence the effect of pH on ionization should be minimal; however, the possibility of a pH-related biological effect exists. Uptake was not affected by various chemical inhibitors or incubation under a nitrogen atmosphere.

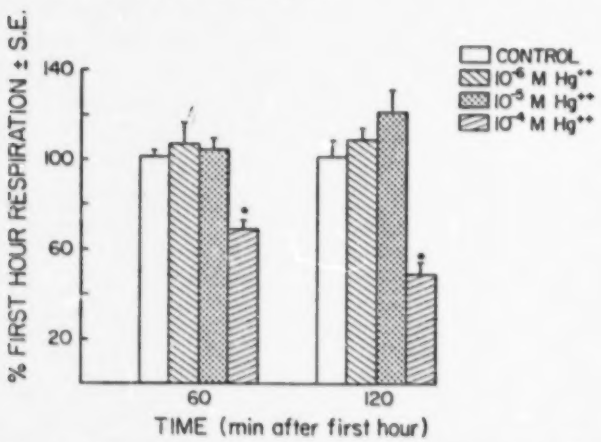

Fig. 1. Effect of mercury on relative oxygen consumption by rat submaxillary slices. Slices were equilibrated for 60 min in medium and then transferred to a separate vial containing metal. Oxygen consumption was determined at 30 min and 60 min during equilibration. Subsequent determinations were expressed as % first hour (equilibration) respiration. Each value represents the mean of 4 determinations. *Significantly different from first hour ($P < 0.05$).

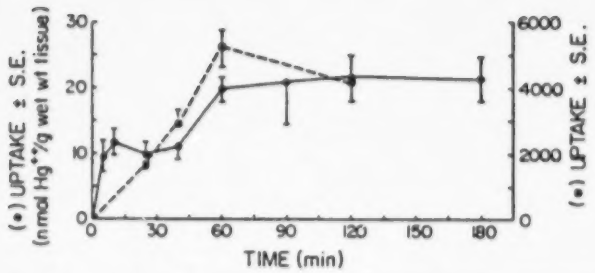

Fig. 2. Uptake of mercury by rat submaxillary slices at various times of incubation. Slices were equilbrated for 60 min in medium and then transferred to a separate vial containing $^{203}Hg^{2+}$. 10^{-6} M Hg^{2+}, ———, left-hand ordinate; 10^{-4} M Hg^{2+}, — — —, right-hand ordinate. Each point represents the mean of 5—27 determinations.

TABLE I

EFFECTS OF VARIOUS INCUBATION CONDITIONS ON MERCURY UPTAKE BY RAT SUBMAXILLARY SLICES

	Uptake ± S.E.	(*n*)[a]
Concentration		
10^{-6} M Hg^{2+} (Control)	19.95 ± 1.64	(27)
10^{-8} M Hg^{2+}	0.133 ± 0.015	(9)[b]
10^{-7} M Hg^{2+}	1.150 ± 0.26	(8)[b]
10^{-5} M Hg^{2+}	648.0 ± 50.1	(10)[b]
10^{-4} M Hg^{2+}	5222 ± 552	(14)[b]
Temperature		
5°C	10.60 ± 2.09	(11)[b]
25°C	16.07 ± 2.05	(10)
pH		
6.8	28.29 ± 3.84	(7)
8.0	19.37 ± 4.49	(7)
Additions		
N_2	23.62 ± 4.21	(8)
10^{-3} M 2,4-DNP	23.31 ± 3.36	(9)
10^{-3} M NaCN	24.59 ± 2.34	(8)
10^{-3} M NaF	18.75 ± 2.84	(8)
10^{-3} M Ouabain	22.48 ± 3.19	(8)
Ca^{2+}-free medium	15.72 ± 1.91	(8)

[a]Expressed as nmol Hg^{2+}/g wet weight tissue; 60-min equilibration, 60-min incubation with $^{203}Hg^{2+}$; standard conditions were 10^{-6} M Hg^{2+} (pH 7.4), 37°C, 100% O_2.
[b]Significantly different from control ($P < 0.05$).

TABLE II

EFFECTS OF VARIOUS METALS ON MERCURY UPTAKE BY RAT SUBMAXILLARY SLICES

	Uptake ± S.E.	$(n)^a$
Control	0.133 ± 0.015	(9)
10^{-4} M $CaCl_2$ [b]	0.193 ± 0.028	(5)
10^{-4} M $CdCl_2$	0.056 ± 0.011	(6)[c]
10^{-4} M $ZnSO_4$	0.159 ± 0.024	(6)
10^{-4} M $CuSO_4$	0.181 ± 0.029	(6)
10^{-4} M $FeCl_3$	0.076 ± 0.029	(7)[c]
10^{-4} M $Fe(NO_3)_3$	0.148 ± 0.019	(6)

[a] Expressed as nmol Hg^{2+}/g wet weight tissue; 60-min equilibration, 60 min incubation with $^{203}Hg^{2+}$; standard conditions were 10^{-8} M Hg^{2+} (pH 7.4), 37°C, 100% O_2.
[b] In addition to the buffer concentration (1.5×10^{-3} M).
[c] Significantly different from control ($P < 0.05$).

Concentrations of Zn^{2+}, Cu^{2+} or Fe^{3+}, 10 000-fold greater than Hg^{2+}, were without effect on 10^{-8} M Hg^{2+} uptake; however, a significant decrease in uptake was produced by 10^{-5} M Fe^{2+} or 10^{-4} M Cd^{2+} (Table II). These experiments were performed with 10^{-8} M $^{203}Hg^{2+}$ as the control condition. When 10 000-fold greater concentrations of metals were added to 10^{-6} M $^{203}Hg^{2+}$, precipitation occurred.

Extrapolation of the slow component of efflux (10^{-6} M $^{203}Hg^{2+}$) to $t = 0$ yielded a value of 81% (16.16 nmol Hg^{2+}/g); 26% of the tissue mercury was removed in 50 min (Fig. 3A). Incubation of slices with a higher concentration of Hg^{2+} (10^{-4} M, Fig. 3B) produced a slower rate of efflux. At $t = 0$, the slow component value was 88% (4596 nmol Hg^{2+}/g); 11% was removed in 50 min. Addition of non-radioactive Hg^{2+} (10^{-4} M, Fig. 3C) to the washout medium produced an immediate and persistent increase in efflux rate.

DISCUSSION

The O_2 consumption results show that slices were viable under standard conditions; high Hg^{2+} concentrations decreased cellular respiration.

The plateau of Hg^{2+} uptake indicates steady-state distribution between tissue and medium metal. Similar behavior was reported in rat renal cortical slices [10].

A proportional increase in mercury uptake was observed with increasing concentration in the medium; this suggests a non-saturable process. Inhibition of oxidative or glycolytic energy production failed to decrease uptake; this reflects an independence of cellular energy. Richardson et al. [10] concluded that Hg^{2+} uptake in the kidney was not dependent on metabolic energy since incubation with DNP caused no depression. However, for Hg^{2+}-cysteine uptake, a metabolic component was evident; the metal-amino acid complex was apparently transported by amino acid mechanisms [11].

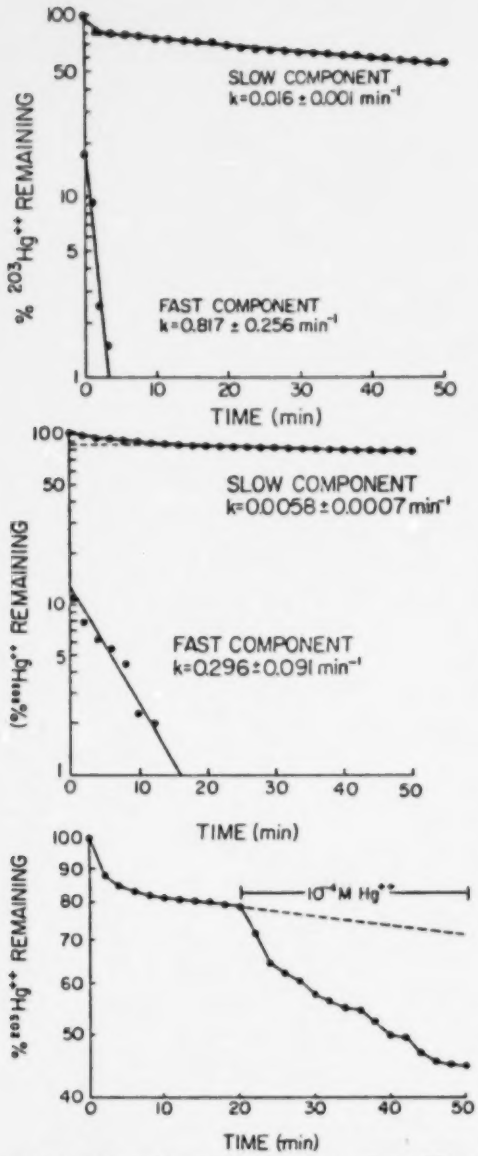

Fig. 3. Efflux of mercury from rat submaxillary slices. Each experimental point represents the mean of 5 determinations. (A) Slices were equilibrated for 60 min in medium, incubated with 10^{-4} M $^{203}Hg^{2+}$ for 60 min and washed out in medium for 50 min in 2-min intervals. The broken line represents the extrapolation of the slow component to zero time. The fast component was obtained by subtracting the percentage values on the extrapolated line from the corresponding experimental values (B) The protocol was the same as for A except slices were incubated with 10^{-4} M $^{203}Hg^{2+}$ prior to washout. (C) The protocol was the same as for A except 10^{-4} M Hg^{2+} was added to the medium at 20 min. The broken line represents the rate of efflux if non-radioactive metal had not been added.

Under the conditions of the current experiments, dissociation of metal salt should not be related to temperature, since the metal is ionized in solution. In addition, changes in pH did not affect uptake. The observed temperature-dependence may reflect: (1) an effect on an active component of mercury uptake, either a minor transport pathway or a process obscured by the absence of secretory units in the slices; or (2) an effect on the binding affinity of a Hg^{2+}-ligand complex.

Ouabin produced no decrease in tissue metal, suggesting that uptake is not dependent upon Na^{+}-K^{+}-ATPase activity. Zn^{2+}, Cu^{2+} and Fe^{3+} failed to affect tissue Hg^{2+} levels (at 10 000-fold greater concentrations) while Fe^{2+} (at a 1000-fold difference) and Cd^{2+} (at a 10 000-fold difference) caused significant decreases. This antagonism may suggest some competition for binding sites, possibly on an iron-transport-like protein.

These observations suggest a passive (simple diffusion) process for uptake.

Firm binding of Hg^{2+} during efflux (after incubation in 10^{-6} M $^{203}Hg^{2+}$) suggested intracellular sites [12]. The appearance of mercury in the saliva of rats intravenously administered mercury, further support intracellular localization [6]. The decreased efflux rate (after incubation in 10^{-4} M $^{203}Hg^{2+}$) may reflect a greater (slow component) uptake into different binding pools (locations) within the cell. The increased rate of washout (with the addition of 10^{-4} M Hg^{2+} after incubation in 10^{-6} M $^{203}Hg^{2+}$) indicates a displacement of bound metal. This may be due to exchange of non-radioactive mercury for radioactive mercury, or it may simply be "poisoning" of the tissue at high concentrations of Hg^{2+} since 10^{-4} M Hg^{2+} produced a significant inhibition of O_2 consumption.

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